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Detection of *Rickettsia* spp. in ring-tailed coatis (*Nasua nasua*) and ticks of the Iguaçu National Park, Brazilian Atlantic Rainforest

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ABSTRACT

Wild animals are of considerable importance in the ecology of infectious agents, as they can function as hosts and even as possible vectors. In this study, DNA from *Rickettsia* spp. was detected on ticks and fragments of skin collected from wild coatis with synanthropic habits in the Iguaçu National Park (INP) in the state of Paraná in southern Brazil. Testing was carried out on a total of 566 ticks, comprising *Amblyomma* spp. larvae, nymphs of *Haemaphysalis juxtakochi, Amblyomma brasiliense, Amblyomma coelebs*, and adults of *Amblyomma ovale*. The samples were tested by polymerase chain reaction (PCR) by amplifying *htrA, gltA, ompA*, and *ompB* gene fragments to detect *Rickettsia* spp. A fragment of each positive sample was sequenced in both directions, submitted to Genbank for a homology search, and also used for phylogenetic analyses. Samples of *A. coelebs* (1.90%, 8/420), *A. ovale* (13%, 6/45), and ring-tailed coati skin (1%, 1/75) amplified *Rickettsia* spp. DNA. Through sequencing, *Rickettsia bellii* was observed in *A. ovale, Rickettsia amblyommatis* in *A. coelebs*, while *Rickettsia rhipicephali* was detected in the skin samples. Wild ring-tailed coatis with synanthropic habits in the INP and their ticks are infected by *Rickettsia* spp., and associations with new hosts have been described.

1. Introduction

The Iguaçu National Park (INP), one of the largest conservation areas in the Brazilian Atlantic Rainforest, covers 185,000 hectares and is home to the Iguaçu Falls, which is one of the natural wonders of the world (Fernandes and Garcia, 2011). The park attracts many Brazilian and foreign visitors every year (ICMBIO, 2020).

The INP's faunal richness includes mammals such as the jaguar, tapir, coati, deer, agouti and peccary, among other species. Coatis (*Nasua nasua*) are carnivores of the Procyonidae family and are widely distributed throughout South America. They are among the species with the greatest synanthropy in the INP visitation areas, especially near the waterfall viewpoint (Brocardo et al., 2019; Caceres, 2011).

Surveys that evaluate the dynamics of wildlife animals infestation by ectoparasites is essential, mainly because it allows knowledge about ticks vectors of pathogens (Spolidorio et al., 2010; Szabó et al., 2013a; Soares et al., 2015). Ticks are important vectors of bacteria of the *Rickettsia* and *Borrelia* genera (Cutler, 2010; Parola et al., 2013) and are frequently found on coatis (Rodrigues et al., 2006; Estevam et al., 2020). However, the importance of coatis as hosts of tick-borne microorganisms, such as *Rickettsia* spp., is still poorly understood.

The genus *Rickettsia* is formed of gram-negative pleomorphic bacteria. It is divided into four groups according to antigenic, morphological, molecular and ecological patterns: the Typhus group, which comprises the species *Rickettsia prowazekii* and *Rickettsia typhi*, associated with lice and fleas, respectively; the spotted fever group, composed of more than 20 species, the vast majority of which are associated with ticks; the *Rickettsia canadensis* group, with *R. canadensis* transmitted by ticks; and the *Rickettsia bellii* group, consisting of *R. bellii* and other symbiotic strains found in ticks (Merhej and Raoult, 2011).

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Bacteria of the genus *Rickettsia* cause at least two rickettsioses in Brazilian territory. Brazilian spotted fever (BSF) is caused by *Rickettsia rickettsii* and transmitted by *Amblyomma aureolatum* and *Amblyomma sculptum* (*Amblyomma cajennense* complex) (Szabó et al., 2013a). A decade ago a milder spotted fever was described by Spolidorio et al. (2010). It was caused by *Rickettsia parkeri* strain Atlantic Rainforest and was transmitted by *Amblyomma ovale* and *A. aureolatum* (Szabó et al., 2013b).

Given the diversity of Brazilian habitats, ticks, and their host species, research is needed to learn more about *Rickettsia* and rickettsioses in the Brazilian territory, especially in wild hosts. In pursuit of this aim, the study described in this paper was conducted to characterize *Rickettsia* spp. in ticks from coatis and in skin samples from wild coatis of synanthropic habits from the INP in the state of Paraná in southern Brazil.

2. Material and methods

2.1. Ethical and legal aspects of scientific research

This research was carried out after approval by the Ethics Committee for the Use of Animals of the Veterinary Institute of the Federal Rural University of Rio de Janeiro (N° 058/2014 CEUA-IV/UFRRJ). The capture of animals, field collection, and transport of biological samples were authorized by the Biodiversity Information and Authorization System (SISBio) of the Ministry of the Environment (N° 43,614–3).

2.2. Study area

This study was carried out at INP, a federal conservation unit managed by the Chico Mendes Institute of Biodiversity (ICMBio). The INP is located in the municipality of Foz do Iguaçu, in the western mesoregion of the state of Paraná in southern Brazil (Fig. 1). It is located within the Atlantic Rainforest biome and covers a total area of 185 thousand hectares. The INP is known for its great wealth of fauna and flora (Fernandes and Garcia, 2011). The INP receives the second-highest number of visitors annually among Brazilian federal conservation units, with more than 2 million tourists from around the world (ICMBIO, 2020).

2.3. Capture of animals and obtaining samples

The ring-tailed coati captures were carried out over a total of 42 days during mornings and afternoons (from 9:00 am to 4:00 pm) in September 2014 and between March and April 2015. The sample number of ring-tailed coatis was defined based on the expected prevalence, using the tick prevalence data obtained by Rodrigues et al. (2006), calculated by the simple random sampling formula and subsequent reduction for finite populations, according to Medronho et al. (2009).

Three collection points were chosen in the tourist area of the INP: points of access to two trails inside the forest (point I - 25°37′36"S, 54°27′39"W and point II - 25°39′05"S, 54°26′16"W) and the viewpoints of the falls (point III - 25°41′03"S, 54°26′24"W, with a total length of approximately 1.2 km) (Fig. 1D).

When sighted, sub-adult and adult ring-tailed coatis were attracted with banana, pineapple, or peanut butter bait and captured with a hand net (multifilament nylon, 60 × 120 cm). Tomahawk traps (90 × 45 × 50 cm and 50 × 21.5 × 20 cm) were also installed throughout the day, one at each collection point. After being contained, the ring-tailed coatis received a pre-anesthesia with 1% atropine (Sulfato de Atropina®, UCB, 1 mg/kg, subcutaneous) and 2% xylazine (Anasedan®, Cespo, 2 mg/kg, intramuscular), except pregnant females, and were then anesthetized with a combination of tiletamine + zolazepan (Zoletil® 50, Virbac, 7 mg/kg, intramuscular).

We collected skin samples to evaluate the potential of ring-tailed coatis to serve as hosts for *Rickettsia* spp. using tissues collected in a minimally invasive manner. A fragment of skin tissue was collected from the posterior edge of the base of the left ear of each animal, which also served as identification to avoid recapture. Before collection, trichotomy and asepsis of the site with 70% ethanol were performed, followed by section with surgical scissors and the aid of tweezers. The fragments were stored in 1.5-mL microtubes with RNAlater® solution and immediately frozen at −20 °C until DNA extraction.

A total of 566 ticks were evaluated in this study, including larvae of *Amblyomma* spp., nymphs of *Haemaphysalis juxtakochi, Amblyomma brasiliense* and *Amblyomma coelebs*, and adults of *Amblyomma ovale*, previously reported by Magalhães-Matos et al. (2017), except for the samples that were deposited in an ixodological collection ("Coleção de Artrópodes Vetores Ápteros de Importância em Saúde das Comunidades" – CAVAISC/ FIOCRUZ). The ectoparasites were stored in RNAlater® and frozen at −20 °C until the moment of molecular analysis.

Information about each animal was recorded on a collection form. Age estimation was performed according to Olifiers et al. (2010), with the ring-tailed coatis classified as puppies (aged up to 6 months), sub-adults (aged 6 months to 2 years), or adults (aged over 2 years).

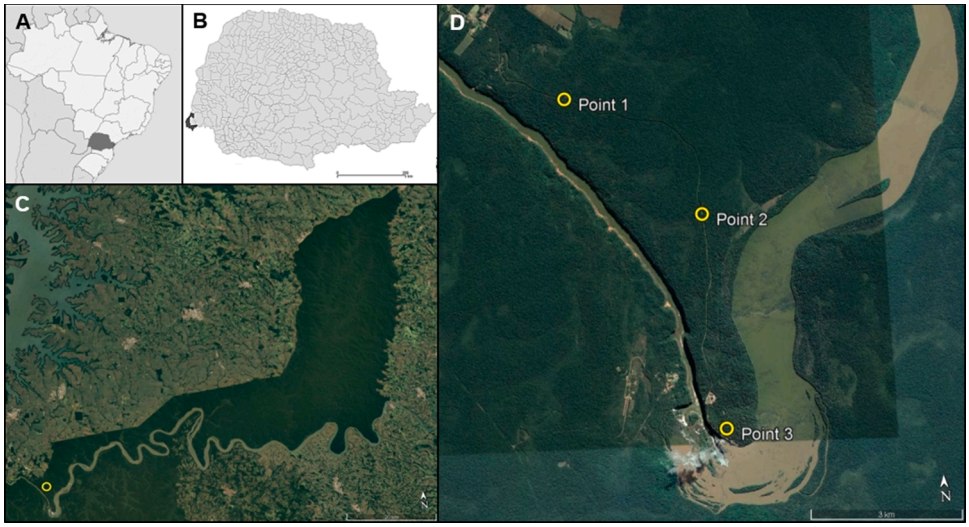

Fig. 1. Study area maps. A) Brazil, with emphasis on the state of Paraná in the southern region; B) State of Paraná, highlighting the municipality of Foz do Iguaçu (scale bar: 200 km); C) Area of the Iguaçu National Park, highlighting the tourist area (point) (scale bar: 20 km); D) Sampling points in the tourist area, along the BR-469 highway (scale bar: 3 km).

After collection, the animals were monitored until they recovered fully from anesthesia, at which time they were reintroduced to their habitat of origin.

2.4. Molecular analysis

Before DNA extraction, all ticks were sequentially washed in a 1% sodium hypochlorite solution, 70% ethanol, and phosphate-buffered saline (PBS). DNA extraction was performed individually from each tick by the phenol–chloroform method, according to the protocol established by McIntosh et al. (2015). A commercial kit (DNeasy® Blood & Tissue, QIAGEN) was used for DNA extraction from ring-tailed coati skin, following the manufacturer's DNA extraction protocol for animal tissues. DNA extracted from ticks and coati skin was quantified, analyzed for purity (NanoDrop™ 2000/2000c Spectrophotometers), and standardized at 50 ng/μL for molecular analysis.

The polymerase chain reaction (PCR) was used to search for *Rickettsia* spp. in tick and skin samples. Primers 17k3 and 17k5 were used for sample screening, which amplify a 549-bp fragment of the *htrA* gene (which encodes a 17 kD membrane antigen) and primers CS-239 and CS-1069, which amplify an 834-bp fragment of the gene *gltA* (which encodes the enzyme citrate synthase), specific for the genus *Rickettsia* (Labruna et al., 2004a). Samples that amplified for both genes evaluated were considered positive.

The screening-positive samples were subjected to two additional PCR protocols. The first used primers Rr190.70p and Rr190.602n, which amplify a 532-pb fragment of the *ompA* gene (which encodes a 190 kD membrane antigen) (Regnery et al., 1991). The second protocol used primers 120-M59 and 120–807, which amplify 856 bp of the *Rickettsia* spp. *ompB* gene (Roux and Raoult, 2000).

For all reactions, the master mix contained 1X buffer (5X Colorless GoTaq®, Promega®), 2.50 mM $MgCl_2$ (Promega® $MgCl_2$ Solution), 0.2 mM dNTP, 10 pmol of each primer, 0.75 U of Taq polymerase (GoTaq DNA Polymerase, Promega®), 100 ng of DNA, and sufficient water to obtain a final volume of 25 μL. The following conditions were used for the PCR reactions: initial denaturation at 95 °C for five min, followed by 40 cycles of 95 °C for 20 s, 52 °C for 20 s and 72 °C for 25 s, with a final extension at 72 °C for 5 min (McIntosh et al., 2015, modified). DNA from *Rickettsia parkeri* strain At24 isolated from *Amblyomma triste* in Paulicéia, São Paulo, Brazil, was used as a positive control of the reactions (Silveira et al., 2007). Ultrapure water applied inside and outside the laminar flow of preparation of the master mix was used as a negative control, in addition to an extraction control (a known negative sample extracted together with the test samples). PCR products were analyzed by gel electrophoresis (1.5% agarose).

The sequencing material was purified with Exo-Sap-IT (GE Healthcare®), following the protocol indicated by the manufacturer. The fragments were sequenced in both directions on an automated genetic analyzer (ABI 3730 DNA Analyzer, Thermo Fisher Scientific®). *Rickettsia* DNA samples in ticks were sequenced for genes *gltA, ompA*, and *ompB*, and the genes *htrA, gltA*, and *ompA* were evaluated in skin samples. The sequences obtained were aligned using the ClustalW program and submitted to homology research with other sequences deposited in GenBank using the BLASTn tool.

2.5. Phylogenetic analysis

The phylogenetic trees were constructed from the fragments of the genes *gltA, ompA*, and *ompB* from samples positive for *Rickettsia* spp. in ticks and ring-tailed coati skin samples. For all phylogenetic analyzes performed, the sequences obtained in the sequencing and those obtained in databases were aligned by the MUSCLE tool (Edgar, 2004) in the Seaview4 program (Gouy et al., 2010). The phylogenetic relationships were estimated using maximum likelihood (MV) phylogenetic inference implemented in the PhyML tool (Guindon and Gascuel, 2003) under a sequence evolution model that was chosen after testing alternative models hierarchically by computation using the Bayesian information criterion, determined in MEGA version 7 (Kumar et al., 2016). The statistical support of clades was measured by a heuristic search with 1000 bootstrap repetitions.

3. Results

A total of 566 tick samples were obtained from 86 animals, while skin samples were collected from 75 ring-tailed coatis. Among these, 49 were females and 26 males; nine were puppies, 33 were sub-adults, and 33 were adults.

Through PCR, 2.47% (14/566) of ticks and 1% (1/75) of ring-tailed coatis amplified *Rickettsia* spp. DNA for the genes evaluated (Table 1).

Of the six samples of *A. ovale* positive in the screening (*htrA* and *gltA* genes), two were from females and four from males of this species, which infested 6% (5/86) of ring-tailed coatis. The result of sequencing the *gltA* gene fragment showed that two of the sequences obtained (5 and 16) showed 100% identity with *Rickettsia bellii* RML369-C (NC_007940), while a third (sample 22) had a 99.8% (601/602) identity with the same strain. Phylogenetic analysis for *gltA* showed that these sequences were in the same clade as *R. bellii*, and sample 22 differed from the others (Fig. 2).

The sequences of the other three *A. ovale* samples (8, 17, and 39) showed 99.3% identity with the *R. bellii* strain RML369-C and were identical to each other. These samples also appeared within the *R. bellii* clade in the dendrogram, but they differed from all other samples. These three samples were considered to be a different strain, defined as the *R. bellii* strain AoNa (Fig. 2). None of the six positive *A. ovale* samples amplified for the *ompA* and *ompB* genes, which was expected because the primers used did not amplify *Rickettsia* DNA from this group. The *R. bellii* sequences deposited in GenBank generated the access codes MZ403758 to MZ403763.

A total of 7% (6/86) of ring-tailed coatis were infested with *A. coelebs* positive for *Rickettsia* spp. The sequencing revealed the homology of the species evaluated with *R. amblyommatis*, being 100% with "*Candidatus* Rickettsia amblyommii" clone Mato Grosso 1 (*ompA* gene, KT722803), 99.6% (*gltA* gene, CP012420) and 97.9% (*ompB* gene, CP012420) with *R. amblyommatis* strain Ac37. The phylogenetic analyses showed that the *R. amblyommatis* detected in this study was in the same clade as other sequences of *R. amblyommatis* (or *R. amblyommii*) deposited in Genbank (Fig. 2 and supplemental files) and was described in this study as *R. amblyommatis* strain Foz. The sequences deposited in GenBank generated the following access codes: *gltA* – MZ403764 to MZ403768; *ompA* – MZ420504 to MZ420511; and *ompB* – MZ420514 to MZ420521.

Only one skin fragment from one of the ring-tailed coatis analyzed was positive for *Rickettsia rhipicephali*. The analyses of the *htrA, gltA*, and

Table 1
Number of samples collected and prevalence of *Rickettsia* spp. per host.

Host species	Stage (ticks)	Number of samples	*Rickettsia* species	Molecular prevalence	
				htrA/ gltA	*ompA/ ompB*
Amblyomma spp.	Larvae	21	–	0%	NE[1]
Haemaphysalis juxtakochi	Nymphs	5	–	0%	NE
Amblyomma brasiliense	Nymphs	75	–	0%	NE
Amblyomma coelebs	Nymphs	420	*Rickettsia amblyommatis*	1.90% (8/ 420)	1.90% (8/ 420)
Amblyomma ovale	Adults	45	*Rickettsia bellii*	13% (6/45)	0%
Nasua nasua (skin)	–	75	*Rickettsia rhipicephali*	1% (1/ 75)	1% (1/ 75)

[1] NE: not evaluated, as it is negative in screening.

Fig. 2. Phylogenetic analysis of *Rickettsia amblyommatis, Rickettsia belli, Rickettsia rhipicephali*, and other *Rickettsia* species based on *gltA* gene sequences using the maximum likelihood method. Tamura 3-parameter + Gamma evolutionary model. Bootstrap: 1000. Bold: present study.

ompA sequences obtained demonstrated 100% homology with sequences from *R. rhipicephali* (*R. rhipicephali* strain RrMG [KX018048] for *htrA* and *gltA; R. rhipicephali* strain HJ#5 [CP013133] for all genes evaluated). Furthermore, the phylogenetic analysis for the *gltA* gene (Fig. 2) revealed that the *R. rhipicephali* 74T strain identified in this study is found in the same clade as *R. rhipicephali* 3–7–6 (U59721). Two specimens of *A. coelebs* that infested this same animal were not PCR positive for *Rickettsia* spp. The animal in question was an adult female with body temperature, heart rate, and respiratory rate within the normal range for the species and no changes exhibited that would be suggestive of any disease. The *R. rhipicephali* sequences deposited in GenBank generated the following access codes: *htrA* – MZ384015; *gltA* – MZ403757; *ompA* – MZ420503. Samples that amplified DNA from *R. rhipicephali* did not produce enough fragments for analysis by sequencing and phylogeny for the *ompB* gene.

4. Discussion

This study is the first that has sought to assess the importance of ring-tailed coatis and their ticks as hosts of *Rickettsia* spp. The DNA of this agent was detected in *A. coelebs* and *A. ovale* ticks. This paper also reports, for the first time, the detection of *R. rhipicephali* DNA in a ring-tailed coati tissue fragment.

In this study, *R. bellii* DNA was detected in *A. ovale* ticks recovered from ring-tailed coatis, making possible the differentiation of two strains. These findings corroborate the information available in the literature because *R. bellii* infection is commonly observed in nature in different species of ixodid ticks, including *A. ovale* (Labruna et al., 2011; Lamattina et al., 2018). In a study conducted with free-living ticks from forest areas in the state of Rondônia, Labruna et al. (2004b) detected prevalences of *R. bellii* ranging from 25.6% to 32.6% in *A. ovale*, higher rates than were observed in the present study.

Rickettsia bellii is a bacterium of unknown pathogenicity, as it has not been detected in humans or other animals. As it is considered to be the most primitive species of the genus, in addition to having a high prevalence in populations of *Amblyomma* spp., possible symbiotic co-evolution processes may have occurred between the host tick and the bacteria (Labruna et al., 2004a; McIntosh et al., 2015). Although it is an agent of unknown pathogenicity, the detection of *R. bellii* in *A. ovale* of INP ring-tailed coatis is an important finding because the presence of a rickettsial agent in the tick can inhibit the transovarial transmission of other rickettsiae (Burgdorfer, 1985), such as *R. rickettsii*, which is highly pathogenic.

Among the six sequences of *R. bellii* evaluated in the present study, two different genotypes of the agent were observed, which indicates that *A. ovale* on ring-tailed coatis of the INP have at least two strains of *R. bellii*. Two sequences were identical to other strains reported in the literature, such as *R. bellii* strain RB-CL detected in *Ixodes loricatus* in Rio Grande do Sul in southern Brazil (Krawczak et al., 2016). A third sequence (sample 22) presented a nucleotide difference from the strains reported in the literature, even standing out in the phylogenetic tree. In relation to the other genotype, three sequences of *R. bellii* presented four different nucleotides from the other samples and from the isolates deposited in Genbank. These sequences belong to a new strain described here as *R. bellii* strain AoNa.

Amplification of *R. bellii* DNA from *A. ovale* in northeastern Argentina, including areas on the Argentine side of the INP, was also a finding by Lamattina et al. (2018), which corroborates the results found at the INP, in Brazil. However, it is important to highlight that these authors also detected DNA of *Rickettsia parkeri* strain Atlantic Rainforest, different from what was observed in the present study. *Amblyomma ovale* is one of the main vectors of *R. parkeri* strain Atlantic Rainforest, a species belonging to the spotted fever group (SFG) and the etiologic agent of a mild rickettsiosis for humans, initially characterized in Brazil

by Spolidorio et al. (2010) and Szabó et al. (2013b). In the present study, DNA from this strain was not detected in the evaluated *A. ovale* ticks, which may be due to the inhibition process mentioned above or because it occurs at very low infection rates in the INP on the Brazilian side, what makes detection difficult. Complementary studies are important to elucidate this issue, especially involving *A. ovale* from other host (wild or domestic around the park) and free ticks in the environment.

The DNA sequence analysis in *A. coelebs* samples showed identity with *R. amblyommatis* strains in all genes evaluated. The diagnosis of *R. amblyommatis* is a common finding in ticks of the genus *Amblyomma* in Brazil, as observed with *Amblyomma auriculatum* (Saraiva et al., 2013), *Amblyomma calcaratum* (Luz et al., 2017), *Amblyomma longirostre* (McIntosh et al., 2015; Krawczak et al., 2016; Luz et al., 2017), and *Amblyomma pacae* (Lopes et al., 2016), for example. Labruna et al. (2004b) detected this agent for the first time in the country in *A. cajennense* sensu lato and *A. coelebs*, even suggesting that the *Rickettsia* sp. strain ARANHA, previously described by them in *A. longirostre* (Labruna et al., 2004a), is actually a strain of *R. amblyommatis* because it is highly related to this species.

The literature demonstrates that the prevalence of *R. amblyommatis* in *A. coelebs* is approximately 10% to 15% (Labruna et al., 2004b; Parola et al., 2007). When researching rickettsial agents in ticks of the genus *Amblyomma* in the state of Rondônia in the Brazilian Amazon, Labruna et al. (2004b) detected a prevalence of 10% (1/10) of infection in evaluated *A. coelebs* and also observed the presence of the agent in 26.8% (11/41) of *A. cajennense*. Parola et al. (2007) detected the agent in 15.4% (2/13) of *A. coelebs* evaluated in French Guiana. The results obtained in this study showed a prevalence of 1.90% (8/420) of *R. amblyommatis* in *A. coelebs*, a value lower than that observed in other studies. This demonstrates that the prevalence of *R. amblyommatis* seems to be lower in *A. coelebs* from the Atlantic Rainforest of southern Brazil than in the Amazon region. It is important to highlight that the sample number of ticks can influence the observed prevalence because more representative samples tend to demonstrate the real prevalence with greater precision in the studied population (Medronho et al., 2009).

In recent years, some new descriptions of pathogens have been carried out in the ring-tailed coatis of Foz do Iguaçu (city of origin of the present study), such as *Mycoplasma* sp., *Mycoplasma haemofelis* (Cubilla et al., 2017), and *Neorickettsia helminthoeca* (Headley et al., 2018). Despite this, no records in the literature report the infection of ring-tailed coatis by species of the genus *Rickettsia*. It is known that the raccoon (*Procyon lotor*), a species of North American procionid, has been found to be seropositive for *R. parkeri* sensu stricto but without molecular evidence of infection (Castellaw et al., 2011). In Brazil, Soares et al. (2015) researched *Rickettsia* DNA in ticks and wild mammals from the Amazon region, including the coati. The authors collected lung and liver samples from ring-tailed coati but did not detect *Rickettsia* DNA by PCR.

In the present study, we chose to collect a skin sample from the ear of each animal, as it was a non-invasive method of obtaining tissue. Skin tissue collection was also chosen because *Rickettsia* spp. cause low bacteremia even in the acute phase of infection, making a blood sample, for example, a less reliable indicator of the real prevalence in the vertebrate host. Skin samples have greater detection sensitivity than blood samples (Levin et al., 2016). In this study, one of the ring-tailed coatis of the INP was found to be positive for *R. rhipicephali*, amplifying fragments of the *htrA, gltA*, and *ompA* genes.

Rickettsia rhipicephali is distributed from North America to South America. In North and Central America, it infects *Rhipicephalus sanguineus, Dermacentor occidentalis, Dermacentor variabilis*, and *Dermacentor andersoni* (Parola et al., 2013). In Brazil, the agent has been detected in *H. juxtakochi* (Labruna et al., 2007a) and, more recently, in *Amblyomma romarioi* (Zeringóta et al., 2017; Martins et al., 2019). *Rickettsia rhipicephali* is considered a bacterium of unknown pathogenicity because, although there are no case reports in humans, intradermal inoculation in guinea pigs induces the formation of inoculation bedsores, suggesting that the agent may also be pathogenic for humans (La Scola et al., 2009; Parola et al., 2013).

This is the first study to detect natural infection by *R. rhipicephali* in a vertebrate animal. However, the agent was not detected in ticks that infested ring-tailed coatis, including in *H. juxtakochi*. Note that only a small population of *H. juxtakochi* ($n = 5$) was obtained from the analyzed ring-tailed coatis, which may have influenced this result. This does not rule out the possibility that *H. juxtakochi* and other species of the genus *Amblyomma* may be functioning as vectors of *R. rhipicephali* for coatis and other wild and domestic species in situ.

Some epidemiological studies have demonstrated the seropositivity of domestic dogs for rickettsial agents in Brazil, including for *R. rhipicephali*, which may demonstrate a role of these animals as hosts of the bacteria (Labruna et al., 2007b; Saito et al., 2008). Coelho et al. (2016) demonstrated seropositivity of small wild mammals for *R. rickettsii, R. parkeri* sensu stricto, *R. bellii*, and *R. rhipicephali*, showing that this agent also circulates in wild populations. Based on this information and the results obtained in this research, it is essential to carry out additional serological studies in ring-tailed coatis to better understand the dynamics of *R. rhipicephali* in these animal populations.

Characteristics such as the large population of ring-tailed coatis with synanthropic habits present in the INP (Brocardo et al., 2019), their ability to disperse in the environment (Beisiegel and Mantovani, 2006), their ability to host vector ticks (Magalhães-Matos et al., 2017), and their contact with other wild and domestic species (in the vicinity of the INP) (Moraes et al., 2017) can make the ring-tailed coati an effective disperser of ticks and the agents transmitted by them. The presence of tourists in the park in the same areas where animals roam is also an important factor that increases the likelihood of transmission of these agents to humans.

5. Conclusion

The ring-tailed coatis of the Iguaçu National Park have ecological importance for the maintenance of ticks infected by *R. amblyommatis* and *R. bellii*. Furthermore, the coatis themselves are infected by *R. rhipicephali*, suggesting that they participate directly and indirectly in the epidemiology of *Rickettsia* spp. in the Iguaçu National Park in Paraná State, Brazil.

Author statement

Authorship contributions

Category 1

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Category 2

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Declaration of Competing Interest

The authors declare no conflicts of interest.

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Supplementary materials

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